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(73) Proprietor: **JAPANESE FOUNDATION FOR CANCER RESEARCH**
37-1, Kamiikebukuro 1-chome
Toshima-ku Tokyo(JP)

(72) Inventor: **Tsuruo, Takashi**
No. 303, 42-4, Yoyogi 5-chome
Shibuya-ku Tokyo(JP)
Inventor: **Hamada, Hirofumi**
No. 206, 46-5, Takinogawa 3-chome
Kita-ku Tokyo(JP)
Inventor: **Sugano, Haruo**
8-13, Minamiogikubo 4-chome
Suginami-ku Tokyo(JP)

(74) Representative: **Fischer, Hans-Jürgen, Dr. et al**
Hoechst AG Zentrale Patentabteilung Postfach 80 03 20
W-6230 Frankfurt am Main 80(DE)

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Description

This invention relates to a monoclonal antibody in relation to drug-resistant cancers and production thereof. More particularly, the present invention relates to the monoclonal antibody, a hybridoma which produces the same and a process for producing the monoclonal antibody.

It has heretofore been observed that cancer cells which are resistant to an antitumor agent selectedly appear upon treatment of the cancer by chemotherapy, which poses a serious problem in the art. It may be an approach to overcoming the problem of drug-resistance to increase the dosage of the antitumor agent. However, increase of dosage will give unnecessary pains to patients through the side effects of disorders to normal cells. Another approach to overcoming the problem of drug-resistance may be to use several types of antitumor agents in combination, but this approach can be accompanied by a problem of what is called pleiotropic drug-resistance, thus bringing about little effect in many cases.

Accordingly, for the purpose of overcoming such drug-resistance of cancer cells, it is an important task to establish a drug or a method which has little side effects, high selectivity and effectiveness against cancer cells exhibiting pleiotropic drug resistance.

A monoclonal antibody which has selectivity to cancer cells having pleiotropic drug-resistance has been already prepared [J. Clin. Oncology, vol. 3, p. 311 ~ 315 (1985)]. It is a monoclonal antibody which is reactive with glycoproteins having molecular weights of 170,000 to 180,000 daltons which appear specifically on the cell membrane of a cancer cell exhibiting pleiotropic drug-resistance. However, the resistant strain used in preparation of this monoclonal antibody is not derived from human but from Chinese hamster, and also nothing is reported about sensitivity of the drug-resistant cancer cells to the drug when use is made of this monoclonal antibody.

Therefore, nobody in the art would believe that the monoclonal antibody according to this prior art can be used in selective treatment of human drug-resistant cancer cells.

It has been found that the monoclonal antibody produced by the hybridoma obtained by fusion between a mous spleen cell immunized with adriamycin-resistant tumor cell and a mouse myeloma cell inhibits selectively growth of a cancer cell which exhibits pleiotropic drug resistance or enhances its sensitivity to the drug.

Accordingly, the monoclonal antibody in relation to drug-resistant cancers is characterized by the definitions (i) to (iv) shown below.

body in relation to drug-resistant cancers as defined below by (i) to (iv) comprises the steps (a) to (g) shown below.

(i) the monoclonal antibody is produced by a hybridoma which is formed as fusion product between a mouse myeloma cell and a spleen cell from a mouse that has been immunized with an adriamycin-resistant K562/ADM strain of a human myelogenous leukemia cell K562;

(ii) the monoclonal antibody is capable of specifically recognizing an adriamycin-resistant strain;

(iii) the antibody is capable of inhibiting growth of an adriamycin-resistant strain or enhancing sensitivity of the strain to vincristine or actinomycin D; and

(iv) the antibody belongs to the IgG isotype.

(a) immunizing a mouse with an adriamycin-resistant K562/ADM strain which has been established from a human myelogenous leukemia cell K562 strain (ATCC CCL 243);

(b) taking spleen cells out of the immunized mouse and preparing a dispersion of the cells;

(c) subjecting the spleen cells together with mouse myeloma cells to cell fusion conditions thereby to prepare a hybridoma as a fusion product between the spleen cell and the myeloma cell;

(d) culturing the mixture of cells obtained from the step (c) on a selective medium on which only the hybridoma can grow;

(e) determining whether the hybridoma-containing supernatant of the medium contains the antibody desired thereby select hybridoma capable of producing the antibody desired;

(f) cloning the hybridoma thus selected; and

(g) incubating the clone in an abdominal cavity of a mouse or on a medium thereby to obtain a monoclonal antibody formed and accumulated in the cancerous ascites or the supernatant of the medium.

The present invention also relates to a hybridoma. That is, the hybridoma capable of producing monoclonal antibody according to the present invention is prepared between a spleen cell obtained from a mouse immunized with an adriamycin-resistant strain K562/ADM of a human myelogenous leukemia cell K562 strain and a mouse myeloma cell.

As mentioned abov and also apparently seen from the exp rimental results as described hereinafter, th monoclonal antibody according to the present invention has the ability of selectively inhibiting growth of a cancer cell xhibiting pleiotropic drug-resistance or enhancing its sen-

Therefore, the monoclonal antibody according to the present invention can be one means for solving the important task of establishing a drug or a method which has little side effects, high selectivity and effectiveness against cancer cells exhibiting pleiotropic drug resistance.

The monoclonal antibody according to the present invention can be produced according to any desired method suited for the purpose comprising production of a hybridoma by cell fusion and production of a monoclonal antibody by this hybridoma, while bearing in mind that the cell for the antigen is an adriamycin-resistant human tumor cell.

Concerning production of monoclonal antibody including the cell fusion method, some reviews and textbooks have been already known and therefore reference should be made to those literatures concerning necessary informations other than the description given below about one example of the present invention. Some suitable literatures may be, for example, G. Galfrè, C. Milstein: Methods Enzymol., vol. 73, p. 3 ~ 46 (1981) and J.W. Goding: Monoclonal Antibodies: Principles and Practice, Academic Press, 1983.

Preparation of hybridoma/monoclonal antibody

(1) Selection and establishment of adriamycin-resistant cancer cell:

The present inventors have already selected and established a strain of human myeloma cell (K562/VCR), resistant to vincristine which is a vincaalkaloid type antitumor agent. This resistant strain has already exhibited a light degree of cross-resistance to adriamycin which is an anthracycline type antitumor agent ["Gann", vol. 74, p. 751 ~ 758 (1983)]. In the example of the present invention, this strain is used as the starting strain. This strain is freely available from the Foundation, Gann Kenkyukai-Cancer Chemotherapeutical Center (Kamiikebukuro 1-37-1, Toshima-ku, Tokyo, Japan).

First, the vincristine-resistant strain K562/VCR is cultured in a RPMI1640 (10% fetal calf serum (FCS)) containing 3 nM of adriamycin which is IC50, and the grown cells are successively cultured in cultural media in which the concentration of adriamycin is increased stepwise by a ratio of about 3-fold to select a drug-resistant strain at high concentration.

The drug-resistant strain selected is grown for about one year under th pres nce of the drug at the maximum concentration at which the resistant strain can grow, and thereafter stable strains which will no longer lose the resistant properties ven when cultured in a medium containing no drug are

(2) preparation of immunized animal spleen cell:

The adriamycin-resistant human myelogenous leukemia cell K562/ADM strain obtained is once washed with 0.5 ml of Hanks' balance buffer saline (hereinafter called HBBS) to 10^7 cells per one mouse, and then suspended in the same amount of HBBS and administered intraperitoneally into a female Balb/c mouse of 4 to 6 weeks old. Administration is continued similarly at the rate of once per week until the antibody valve rises sufficiently, and three days before cell fusion, 10^6 cells suspended in 0.1 ml are administered intravenously as the booster. From the immunized animal thus obtained, the spleen is collected aseptically. The spleen taken out is loosened aseptically on a laboratory dish by means of a pair of tweezers, and the number of cells obtained is calculated by sampling a part thereof.

For assaying of antibody value, the anti-adriamycin resistant strain antibody value is examined by enzyme immunoassay according to the solid phase method as described below.

① Pretreatment of plate: on Falcon plate 3912, 50 µl of 0.001% poly-L-lysine solution is added per well and after incubation at room temperature for 30 minutes, water is drained, followed by drying on air.

② Binding of cell to plate: K562 parent strain and K562/ADM resistant strain are each suspended at a concentration of 2,000,000 cells/ml and apportioned each in 50 µl (100,000 cells) into the respective well, and the cells are bound to the well by centrifugation at 1000 rpm for 5 minutes. Then, binding is made more perfect by addition of 50 µl of 0.5% glutalaldehyde per well.

③ Blocking: for masking of superfluous binding groups, 200 µl of bovine serum albumin (BSA) dissolved to 3% in RPMI1640 was added per well and the treatment is carried out at room temperature for 30 minutes.

④ Addition of sample to be assayed: each 50 µl of the sample for assay of antibody value is added to one well, and K562 and K562/ADM are added respectively for one kind of sample, and incubation is performed at room temperature or 37°C for 2 hours. Then, washing is repeated 4 times with phosphat buffer saline (PBS).

⑤ Addition of secondary antibody: as the secondary antibody, F $(ab')_2$ fragment of the peroxidase bound-antimous Ig antibody of goat [produced by Cappel Co., U.S.A.], which is diluted into a solution to 1,500-fold in PBS, is added into each well and incubation is further performed at room temp rature for 2 hours.

⑥ Judgement: as the substrat for peroxidase,

after termination of the reaction with sulfuric acid, presenc of and difference in color formation between K562 and K562/ADM are judged with naked eyes or an autoreader.

(3) Preparation of mouse myeloma cell:

As the myeloma strain, for example, 8-azaguanine-resistant myeloma strain P3•X63•Ag8•653 derived from mouse [Journal of Immunology, vol. 123, p. 1548 ~ 1550 (1979)] is used. On the day of fusion, 2 x 10^7 or more cells should be ready for use. This strain is registered as CRL-1580 at American Type Culture Collection (Maryland, U.S.A.) and is freely available therefrom or from Flow Laboratory Inc., U.S.A.

(4) Cell fusion:

The spleen cell obtained from the immunized animal in (2) and the myeloma cell obtained in (3) are mixed so that the number of cells may be spleen cell: myeloma cell = 7:1, and cell fusion is effected in a RPMI-1640 medium containing 43% of polyethyleneglycol 4000 and 13% of dimethylsulfoxide.

The fused cell is grown on a 96-well plastic plate in a RPMI-1640 medium containing hypoxantine, aminopterin and thymidine (hereinafter abbreviated as HAT) for 7 days, and further in a medium containing no HAT. During this cultivation, the medium is exchanged with new one every 3 to 5 days.

About two weeks after fusion, an examination is carried out for the cells survived thereby to check the presence of antibody capable of binding selectively to K562/ADM in the culture supernatant, according to the enzyme immunoassay shown in the above (2).

For positive wells, cloning of positive cells is performed by repeating the limiting dilution method with the use of a RPMI-1640 containing 20% fetal calf serum as the diluting solution.

(5) preparation of monoclonal antibody:

To a mouse which has been previously pretreated by administering intraperitoneally 0.5 ml of pristan per mouse, 10^7 hybridoma cells producing the desired antibody are administered intraperitoneally. Then ascite canceration of hybridoma occurs about two w ks aft r administration, and th ascite built up is collected and pr sence of the antibodies accumulated is examined by the enzym immunoassay of the above (2).

When the ascite is to be stored, the supernatant after centrifugal separation is divided into

For further purification of the antibody, the ascite is salted out at 4•C with 45% saturated ammonium sulfat , followed further by gel filtration by use of®Sephacryl-400 (Pharmacia Co., Sweden). Quantitative determination of protein is carried out by the Rowry method.

PROPERTIES OF MONOCLONAL ANTIBODY

The class of the monoclonal antibody obtained as described above can be judged by use of the antibodies of the respective classes of antimous Ig of rabbit (produced by Cappel Co., U.S.A.).

On the other hand, local existence of the antigen in cell can be known by observation of fluorescence under a microscope by use of a goat antimouse Ig antibody bound with FITC which is the fluorescent chromogenic group (produced by Cappel Co., U.S.A.).

Selectivity for K562/ADM of drug-resistant strain can be examined according to the enzyme immunoassay of the above (2) with the use of the parent strain K562 as a control, or otherwise by measuring the extent of inhibition of cell growth against the respective strains. The change of the sensitivity of the drug-resistant strain to a drug with the monoclonal antibody can be examined also by measuring the extent of inhibition of the cell growth by permitting the strain to be co-present with the monoclonal antibody in the presence of various concentrations of the drug.

Typical examples of the monoclonal antibodies according to the present invention include those of which isotype is IgG_3, IgG_{2a} and IgG_1, etc. In the present invention, these are named as MRK4, MRK16 and MRK17, respectively. Among them, those representative are MRK16 and MRK17.

The monoclonal antibodies MRK16 and MRK17 have the activity of selective growth inhibition against drug-resistant human cancer cells or the activity of increasing sensitivity to drugs (see Example II).

EXPERIMENTAL EXAMPLES

Example I Preparation of monoclonal antibody

a) Selection and stablishment of adriamycin-resistant strain:

For selection of an adriamycin-resistant strain K562/ADM, a vincristine-r sistant strain K562/VCR which had been prepared from the same parent strain of a human myelogenous leukemia cell K562 strain was used as the starting strain.

First, K562/VCR was cultured in a RPMI-1640 (containing 10% fetal calf serum) containing 15 nM

inhibits 50% of cell growth) for this strain for one week, and thereafter the cells grown wer selected. Next, the drug concentration of adriamycin was increased to about 3-fold of 50 nM and cultivation was carried out at that concentration for one week. The resistant cells obtained were further cultured at a drug concentration which was increased stepwise to 150 nM which was the 3-fold amount, until finally adriamycin-resistant cells capable of growing even in the presence of 450 nM of adriamycin could be obtained.

The adriamycin-resistant cells were continuously cultured in a medium containing 500 nM of this drug for about one year, whereby they became stable resistant strains which would not lose the resistance even when cultured in a medium containing no adriamycin for about 3 months thereafter. The present inventors named this strain "K562/ADM".

b) Immunization of mouse and cell fusion:

For the K562/ADM obtained in a), an amount corresponding to 10^7 cells per mouse was suspended in 0.5 ml of HBBS and, after washing once, administered intraperitoneally into a female Balb/c mouse of 4 to 6 weeks old. Similarly, administration was continuously repeated once per week over 6 weeks and, in the last week, 10^6 cells as the booster were suspended in 0.1 ml of HBSS and intravenously administered. Three days later, the spleen was aseptically taken out. The spleen cells were obtained in numbers of 1.4 to 3 x 10^8 per mouse. The spleen was loosened by a pair of tweezers and made into a suspension.

Cell fusion was practiced following the method of Kohler & Milstein. More specifically, 1.4 x 10^8 of the spleen cells were fused with 2 x 10^7 of p3•63•Ag8•653 myeloma cells in a RPMI 1640 medium containing 43% polyethyleneglycol 4000 and 13% dimethylsulfoxide.

c) Selection of hybridoma:

After cell fusion, the fused cells were grown in HAT medium on a 96-well plastic plate for 7 days and further in a medium containing no HAT for 7 days. Culturing of about 300 to 400 wells per mouse was conducted and growth of cells could be seen in about 75% of th wells. For the culture supernatant of these wells, the reactivities with K562 and K562/ADM were examined according to enzyme immunoassay. As a result, in about 2/3 of the wells, positive color formations of the same extent could be s en in both of K562 and K562/ADM while no reaction with both occurred in the remaining 1/3 of the wells. Further, wells were K562/ADM per 300 to 400 wells derived from one mouse, but apparently reacted more strongly with K562/ADM.

After screening of about 6,000 wells by use of 18 mice in all, 25 wells having the same reactivity of K562<K562/ADM were found.

d) Cloning of hybridoma:

For 25 wells selected in c), the reaction positive cells were cloned according to the limiting dilution method. As the diluting solution, a RPMI-1640 containing 20% fetal calf serum was used and culturing was carried out with dilution so that 0.5 to 5 cells were partitioned per well. For each well, presence and the properties of the antibody in the culture supernatant were examined according to enzyme immunoassay. For strongly positive wells, cloning according to the limiting dilution method was further repeated, whereby clones of 25 stable hybridomas could be obtained.

e) Production and purification of antibody:

For 16 clones of the 25 hybridomas obtained in d), 10^7/mouse were administered intraperitoneally into mouse to generate ascites cancer, and 5 to 10 ml of ascites per mouse was obtained two weeks later. The remaining 9 clones were stored under freezing (at -70•C). The total protein content in ascites was found to be 5 to 15 mg/ml.

Purification of the antibody was carried out by salting out these ascites with 45% saturated ammonium sulfate, followed by gel filtration with Sephacryl-400 (produced by Pharmacia, Sweden).

Example II Properties of monoclonal antibodies MRK16 and MRK17

a) Isotype of antibody:

For the 16 kinds of the monoclonal antibodies obtained, their isotypes were determined by means of a kit using the antibodies of respective isotypes of rabbit anti-mouse Ig produced by Cappel Co., U.S.A. As a result, it was found that one IgG_3 - (MRK4), one IgG_{2a} (MRK16) and one IgG_1 - (MRK17) were obtained, all of the others belonging to IgM isotype.

b) Judgement of antigenic site according to the fluorescent antibody method:

By use of FITC-bound goat anti-mouse Ig antibody (Cappel Co.), the binding sit s of the r spective monoclonal antibodies of MRK16 and MRK17 with the antigen on K562/ADM cell were xamined.

tion distributed in a ring was observed on cell membrane surface. That is, either one of the antibodies was found to recognize the antigen existing locally on the membrane of the K562/ADM cell. When the same treatment was carried out on the parent strain of K562 as control, no such fluorescent color formation could be seen at all. Thus, it has been found that both MRK16 and MRK17 have strong selectivities for K562/ADM. Also, according to the radioimmunoassay, it has been found that either MRK16 or MRK17 does not react with K562 cells but reacts with extremely high selectivity with K562/ADM which is the resistant strain (see Fig. 1). More specifically, 10^6 cells were allowed to react respectively with ascites diluted solutions of MRK16 and 17 and, after washing the cells, the cells were incubated with F $(ab')_2$ fragment of sheep antimouse Ig labelled with ^{125}I (produced by Amersham Co., U.K.) at 4°C for 30 minutes. After washing, the reactivities (cpm) of MRK16 and 17 which had reacted with the cells were measured to obtain the results as shown in Fig. 1.

c) Discrimination of other drug-resistant strains:

Heretofore, only a few number of adriamycin-resistant strains have been found in human tumor strains. For the only available adriamycin-resistant strain 2780^{AD} of a human ovary carcinoma strain 2780, the reactivities with MRK16 and MRK17 were examined by use of radioimmunoassay. As a result, both of the monoclonal antibodies were found to react more strongly with the resistant strain of 2780^{AD} as compared with the parent strain of 2780 (see Fig. 2). More specifically, 3×10^5 cells were put on a dish, and 24 hours later when the cells were already adhered onto the dish, and the reactivities (cpm) of MRK16 and 17 were measured similarly as in the case shown in Fig. 1 to obtain the results as shown in Fig. 2.

d) Selective cell growth inhibition of MRK17 against K562/ADM:

For the resistant strain K562/ADM and its parent strain K562, and also for the resistant strain 2780^{AD} belonging to another train and its parent strain 2780 for comparative purpose, an ascites containing the monoclonal antibody MRK17 was diluted stepwise for examination of the inhibitory effect against cell growth, respectively (see Fig. 3). Mor specifically, 2×10^4 K562 and K562/ADM c lls, and 6×10^4 2780 and 2780^{AD} cells wer incubated together with th ascites diluted solution of MRK17, and the cell number after 72 hours was measured and the percent growth inhibitions calculated therefrom to obtain th results as shown in

As a result, it was found that substantially no inhibition could be seen against th respectiv sensitive parent strains of K562 or 2780 even when the concentration of the antibody was increased to dilution of 1/400, while about 30% growth inhibition could be seen in the drug-resistant strain of 2780^{AD} at the same antibody concentration, and 80% growth inhibition with K562/ADM, and even 90% or higher growth inhibition at the diluted antibody of 1/100 in the case of K562/ADM were observed. (triplicate data SD<4%)

When the same test was conducted by use of MRK16, a light degree of growth inhibition of about 12% at the maximum could be seen against K562/ADM.

Thus, the great characteristic feature of MRK17 can be said to have a potent and selective growth inhibition activity for drug-resistant strains.

e) Effect on drug-sensitivity of K562/ADM by MRK16:

In the above d), MRK16 was found to have a light degree of direct growth inhibition effect against the drug-resistant strain K562/ADM. When vincristine was added at a concentration of 0.5 μg/ml to this experiment ratio, however, growth inhibition against the resistant strain up to about 75% at the maximum was observed by the co-presence of MRK16 (see Fig. 4). More specifically, 2×10^4 K562/ADM cells either in the presence or absence of 500 ng/ml of vincristine (VCR) were incubated together with ascites diluted solutions of MRK16, and the growth inhibition percents after 72 hours were measured to obtain the results as shown in Fig. 4.

When the effect of MRK16 for accumulation in K562/VCR cells by use of vincristine was examined, an increase by about 60 to 70% as compared with the control test without addition of the antibody could be seen (see Fig. 5). More specifically, 2×10^5 K562/VCR cells either in the presence or absence of MRK16 (ascites diluted to 1:200) were incubated together with [^3H] VCR (100 nM) and the vincristine incorporated within the cell was quantitated to obtain the results as shown in Fig. 5.

Thus, MRK16 has the possibility of recognizing the acting site which participates in expelling intracellular drugs out of the cells which are more advanced in resistant strains.

Here, when th change in sensitivity to vincristine was examined by use of 2780^{AD} which is a resistant strain other than K562/ADM, reduction by 20 to 30% in terms of IC50 value, namely increase of sensitivity to the drug could b seen.

Also, other than vincristine, MRK16 could potentiate the sensitivity of K562/ADM to ac-

specifically, 2 x 10^4 K562/ADM cells either in the presence or absence of MRK16 (ascites diluted to 1:1000) were incubated at 37°C together with various concentrations of actinomycin D, and the growth inhibitory percents after 72 hours were measured to obtain the results as shown in Fig. 6.

From these results, it can be said that MRK16 can act selectively on a large number of anticancer drug-resistant strains to enhance sensitivity to the drug.

BRIEF DESCRIPTION OF THE DRAWINGS:

Figs. 1(A) and (B) are graphs showing reactivities, upon radioimmunoassay, of MRK16(A) and MRK17(B) with human myelogenous leukemia cells K562 and its adriamycin-resistant strain of K562/ADM cells;

Figs. 2(A) and (B) are graphs showing reactivities, upon radioimmunoassay, of MRK16(A) and MRK17(B) with human ovary cancer cells 2780 and with the adriamycin-resistant strain of the cells 2780, namely 2780^{AD};

Fig. 3 is a graph showing cell growth inhibition by MRK17;

Fig. 4 is a graph showing cell growth inhibition by a combination of MRK16 and vincristine;

Fig. 5 is a graph showing uptake of vincristine by MRK16; and

Fig. 6 is a graph showing enhancement of sensitivity to actinomycin D by MRK16.

Claims

Claims for the following Contracting States : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. A monoclonal antibody in relation to drug-resistant cancers characterized by:
   (i) that the monoclonal antibody is produced by a hybridoma which is formed as fusion product between a mouse myeloma cell and a spleen cell from a mouse that has been immunized with an adriamycin-resistant K562/ADM strain of a human myelogenous leukemia cell K562;
   (ii) that the monoclonal antibody is capable of specifically recognizing an adriamycin-resistant strain;
   (iii) that the antibody is capable of inhibiting growth of an adriamycin-resistant strain or enhancing sensitivity of the strain to vincristine or actinomycin D; and
   (iv) that the antibody belongs to the IgG isotype.

2. A process for producing a monoclonal antibody in relation to drug-resistant cancers characterized by:
   (i) that the monoclonal antibody is produced by a hybridoma which is formed as fusion product between a mouse myeloma cell and a spleen cell from a mouse that has been immunized with an adriamycin-resistant K562/ADM strain of a human myelogenous leukemia cell K562;
   (ii) that the monoclonal antibody is capable of specifically recognizing an adriamycin-resistant strain;
   (iii) that the antibody is capable of inhibiting growth of an adriamycin-resistant strain or enhancing sensitivity of the strain to vincristine or actinomycin D; and
   (iv) that the antibody belongs to the IgG isotype; and the process comprises the steps of:
   (a) immunizing a mouse with an adriamycin-resistant K562/ADM strain which has been established from a human myelogenous leukemia cell K562 strain;
   (b) taking spleen cells out of the immunized mouse and preparing a dispersion of the cells;
   (c) subjecting the spleen cells together with mouse myeloma cells to cell fusion conditions thereby to prepare a hybridoma as a fusion product between the spleen cell and the myeloma cell;
   (d) culturing the mixture of cells obtained from the step (c) on a selective medium on which only the hybridoma can grow;
   (e) determining whether the hybridoma-containing supernatant of the medium contains the antibody desired thereby to select hybridoma capable of producing the antibody desired;
   (f) cloning the hybridoma thus selected; and
   (g) incubating the clone in an abdominal cavity of a mouse or on a medium thereby to obtain a monoclonal antibody formed and accumulated in the cancerous ascites or the supernatant of the medium.

3. A hybridoma capable of producing a monoclonal antibody according to claim 1, which hybridoma is formed as fusion product between a mouse myeloma cell and a spleen cell from a mouse that has been immunized with an adriamycin-resistant K562/ADM strain of a human myelogenous leukemia cell K562.

Claims for the following Contracting State : AT

1. A process for preparing a monoclonal antibody in relation to drug-resistant cancers which
   (a) is capable of specifically recognizing an adriamycin-resistant strain;
   (b) is capable of inhibiting growth of an adriamycin-resistant strain or enhancing sensitivity of the strain to vincristine or actinomycin D; and
   (c) belongs to the IgG isotype, characterized by fusing a mouse myeloma cell and a spleen cell from a mouse that has been immunized with an adriamycin-resistant K562/ADM strain of a human myelogenous leukemia cell K562, culturing the hybridoma fusion product and isolating the antibody.

2. A process as claimed in claim 1, which comprises the steps of:
   (a) immunizing a mouse with an adriamycin-resistant K562/ADM strain which has been established from a human myelogenous leukemia cell K562 strain;
   (b) taking spleen cells out of the immunized mouse and preparing a dispersion of the cells;
   (c) subjecting the spleen cells together with mouse myeloma cells to cell fusion conditions thereby to prepare a hybridoma as a fusion product between the spleen cell and the myeloma cell;
   (d) culturing the mixture of cells obtained from the step (c) on a selective medium on which only the hybridoma can grow;
   (e) determining whether the hybridoma-containing supernatant of the medium contains the antibody desired thereby to select hybridoma capable of producing the antibody desired;
   (f) cloning the hybridoma thus selected; and
   (g) incubating the clone in an abdominal cavity of a mouse or on a medium thereby to obtain a monoclonal antibody formed and accumulated in the cancerous ascites or the supernatant of the medium.

3. A process for preparing a hybridoma capable of producing a monoclonal antibody as defined in claim 1, which comprises fusing a mouse myeloma cell and a spleen cell from a mouse that has been immunized with an adriamycin-resistant K562/ADM strain of a human myelogenous leukemia cell K562.

Revendications

Revendications pour les Etats contractants suivants : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

résistants aux médicaments caractérisé en ce que:
   (i) l'anticorps monoclonal est produit par un hybridome issu de la fusion entre une cellule de myélome murin et une cellule de rate provenant d'une souris immunisée au préalable par une lignée K562/ADM résistante à l'adriamycine dérivée d'une lignée K562 de cellules de leucémie myéloïde humaine;
   (ii) l'anticorps monoclonal est capable de reconnaître d'une manière spécifique une lignée résistante à l'adriamycine;
   (iii) l'anticorps est capable d'inhiber la prolifération d'une lignée résistante à l'adriamycine ou d'accroître sa sensibilité à la vincristine ou à l'actinomycine D; et
   (iv) l'anticorps appartient à l'isotype IgG.

2. Procédé de production d'un anticorps monoclonal ayant trait aux cancers résistants aux médicaments caractérisé en ce que:
   (i) l'anticorps monoclonal est produit par un hybridome issu de la fusion entre une cellule de myélome murin et une cellule de rate provenant d'une souris immunisée au préalable par une lignée K562/ADM résistante à l'adriamycine dérivée d'une lignée K562 de cellules de leucémie myéloïde humaine;
   (ii) l'anticorps monoclonal est capable de reconnaître d'une manière spécifique une lignée résistante à l'adriamycine;
   (iii) l'anticorps est capable d'inhiber la prolifération d'une lignée résistante à l'adriamycine ou d'accroître sa sensibilité à la vincristine ou à l'actinomycine D; et
   (iv) l'anticorps appartient à l'isotype IgG et le procédé comporte les étapes suivantes:
      (a) immuniser une souris avec une lignée K562/ADM résistante à l'adriamycine qui a été établie à partir d'une lignée K562 de cellules de leucémie myéloïde humaine;
      (b) prélever des cellules de rate sur l'animal immunisé et préparer une dispersion cellulaire;
      (c) placer des cellules de rate et des cellules de myélome murin dans des conditions de fusion cellulaire dans le but de préparer un hybridome issu de la fusion entre une cellule de rate et une cellule de myélome;
      (d) cultiver le mélanges cellulaires obtenus à l'étape (c) sur un mili u sélectif sur lequel seul l'hybridome peut proliférer;
      ( ) déterminer si l'anticorps désiré se trouve dans le surnageant contenant l'hybridome afin de sélectionner l'hybridome

(f) cloner l'hybridome ainsi sélectionné; et

(g) incuber le clone dans la cavité abdominale d'une souris ou sur un milieu afin d'obtenir un anticorps monoclonal formé et accumulé dans les ascites cancéreux ou dans la surnageant du milieu

3. Hybridome capable de produire un anticorps monoclonal selon la revendication 1, lequel hybridome est issu de la fusion entre une cellule de myélome murin et une cellule de rate provenant d'une souris immunisée au préalable par une lignée K562/ADM résistante à l'adriamycine dérivée d'une lignée K562 de cellules de leucémie myéloïde humaine.

Revendications pour l'Etat contractant suivant : AT

1. Procédé de production d'un anticorps monoclonal ayant trait aux cancers résistants aux médicaments qui:

(a) est capable de reconnaître d'une manière spécifique une lignée résistante à l'adriamycine;

(b) est capable d'inhiber la prolifération d'une lignée résistante à l'adriamycine ou d'accroître sa sensibilité à la vincristine ou à l'actinomycine D; et

(c) appartient à l'isotype IgG, caractérisé par la fusion d'une cellule de myélome murin et d'une cellule de rate provenant d'une souris immunisée au préalable par une lignée K562/ADM résistante à l'adriamycine dérivée d'une lignée K562 de cellules de leucémie myéloïde humaine, la culture de l'hybridome issu de la fusion et la purification de l'anticorps.

2. Procédé selon la revendication 1, qui comporte les étapes suivantes:

(a) immuniser une souris avec une lignée K562/ADM résistante à l'adriamycine qui a été établie à partir d'une lignée K562 de cellules de leucémie myéloïde humaine;

(b) prélever des cellules de rate sur l'animal immunisé et préparer une dispersion cellulaire;

(c) placer des cellules de rate et des cellules de myélome murin dans des conditions de fusion cellulaire dans le but de préparer un hybridom issu de la fusion ntre une cellule de rate et une c llule de myélome;

(d) cultiver le mélanges cellulaires obtenus à l'étape (c) sur un milieu sélectif sur lequel seul l'hybridome peut proliférer;

dans le surnageant contenant l'hybridome afin de sélectionner l'hybridome capable de produire l'anticorps désiré;

(f) cloner l'hybridome ainsi sélectionné; et

(g) incuber le clone dans la cavité abdominale d'une souris ou sur un milieu afin d'obtenir un anticorps monoclonal formé et accumulé dans les ascites cancéreux ou dans le surnageant du milieu

3. Procédé de préparation d'un hybridome capable de produire un anticorps monoclonal selon la revendication 1, qui comporte la fusion d'une cellule de myélome murin et d'une cellule de rate provenant d'une souris immunisée au préalable par une lignée K562/ADM résistante à l'adriamycine dérivée d'une lignée K562 de cellules de leucémie myéloïde humaine.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Monoklonaler Antikörper in bezug auf arzneimittelresistente Krebse, dadurch gekennzeichnet, daß:

(i) der monoklonale Antikörper von einem Hybridom produziert wird, des als Fusionsprodukt zwischen einer Mäuse-Myelomzelle und einer Milzzelle aus einer Maus gebildet worden ist, welche Maus mit einem Adriamycin-resistenten K562/ADM-Stamm einer myelogenen Human-Leukämiezelle K562 immunisiert worden ist;

(ii) der monoklonale Antikörper befähigt ist, einen Adriamycin-resistenten Stamm spezifisch zu erkennen;

(iii) der Antikörper befähigt ist, das Wachstum eines Adriamycin-resistenten Stammes zu hemmen oder die Sensitivität des Stammes gegenüber Vincristin oder Actinomycin D zu erhöhen; und daß

(iv) der Antikörper dem IgG-Isotypus angehört.

2. Verfahren zur Herstellung eines monoklonalen Antikörpers in bezug auf arzneimittelresistente Krebse, dadurch gekennzeichnet, daß der Antikörper dadurch gekennzeichnet ist, daß:

(i) der monoklonal Antikörper von einem Hybridom produziert wird, des als Fusionsprodukt zwischen einer Mäuse-My lomzelle und einer Milzzelle aus einer Maus gebildet worden ist, welche Maus mit einem Adriamycin-r sistenten K562/ADM-Stramm einer myelogenen Human-Leukämiez lle

(ii) der monoklonale Antikörper befähigt ist, einen Adriamycin-resistenten Stamm spezifisch zu _rkenn_n;
(iii) der Antikörper befähigt ist, das Wachstum eines Adriamycin-resistenten Stammes zu hemmen oder die Sensitivität des Stammes gegenüber Vincristin oder Actinomycin D zu erhöhen; und daß
(iv) der Antikörper dem IgG-Isotypus angehört; und daß das Verfahren die folgenden Stufen umfaßt:

(a) Immunisieren einer Maus mit einem Adriamycin-resistenten K562/ADM-Stamm, der aus einem myelogenen Human-Leukämiezellen-K562-Stamm erhalten worden ist;
(b) Entnehmen von Milzzellen aus der immunisierten Maus und Herstellen einer Zelldispersion;
(c) Einwirkenlasssen von Zellfusionsbedingungen auf die Milzzellen zusammen mit Mäuse-Myelomzellen zur Herstellung eines Hybridoms als Fusionsprodukt zwischen der Milzzelle und der Myelomzelle;
(d) Kultivieren des in der Stufe (c) erhaltenen Gemisches von Zellen auf einem selektiven Medium, auf welchem nur das Hybridom wachsen kann;
(e) Feststellen, ob der das Hybridom enthaltende Überstand des Mediums den gewünschten Antikörper enthält und dadurch Auswählen eines Hybridoms, welches befähigt ist, den gewünschten Antikörper zu produzieren;
(f) Klonieren des so ausgewählten Hybridoms; und
(g) Inkubieren des Klons in der Bauchhöhle einer Maus oder auf einem Medium, um dadurch einen monoklonalen Antikörper zu erhalten, der in dem krebsigen Aszites oder in dem Überstand des Mediums gebildet worden ist und sich dort angesammelt hat.

3. Hybridom, welches befähigt ist, einen monoklonalen Antikörper gemäß Anspruch 1 zu produzieren, und welches Hybridom als Fusionsprodukt zwischen einer Mäuse-Myelomzelle und einer Milzzelle aus einer Maus gebildet worden ist, welche Maus mit einem Adriamycin-resistent n K562/ADM-Stamm i-ner myelogenen Human-Leukämiezelle-K562 immunisiert worden ist.

Patentansprüche für folgenden Vertragsstaat : AT

1. Verfahren zur Herstellung eine monoklonalen Antikörpers in bezug auf arzneimittelresistente Krebse, welcher Antikörper
(a) befähigt ist, einen Adriamycin-resistenten Stamm spezifisch zu erkennen;
(b) befähigt ist, das Wachstum eines Adriamycin-resistenten Stammes zu hemmen oder die Sensitivität des Stammes gegenüber Vincristin oder Actinomycin D zu erhöhen; und
(c) dem IgG-Isotypus angehört, gekennzeichnet durch Fusionieren einer Mäuse-Myelomzelle und einer Milzzelle aus einer Maus, welche Maus mit einem Adriamycin-resistenten K562/ADM-Stamm einer myelogenen Human-Leukämiezelle K562 immunisiert worden ist, Kultivieren des Hybridomen-Fusionsproduktes und Isolieren des Antikörpers.

2. Verfahren nach Anspruch 1, welches die folgenden Stufen umfaßt:
(a) Immunisieren einer Maus mit einem Adriamycin-resistenten K562/ADM-Stamm, der aus einem myelogenen Human-Leukämiezellen-K562-Stamm erhalten worden ist;
(b) Entnehmen von Milzzellen aus der immunisierten Maus und Herstellen einer Zelldispersion;
(c) Einwirkenlasssen von Zellfusionsbedingungen auf die Milzzellen zusammen mit Mäuse-Myelomzellen zur Herstellung eines Hybridoms als Fusionsprodukt zwischen der Milzzelle und der Myelomzelle;
(d) Kultivieren des in der Stufe (c) erhaltenen Gemisches von Zellen auf einem selektiven Medium, auf welchem nur das Hybridom wachsen kann;
(e) Feststellen, ob der das Hybridom enthaltende Überstand des Mediums den gewünschten Antikörper enthält und dadurch Auswählen eines Hybridoms, welches befähigt ist, den gewünschten Antikörper zu produzieren;
(f) Klonieren des so ausgewählten Hybridoms; und
(g) Inkubieren des Klons in der Bauchhöhle einer Maus oder auf einem Medium, um dadurch einen monoklonalen Antikörper zu erhalten, der in dem krebsigen Aszites oder in dem Überstand des Mediums gebildet worden ist und sich dort angesammelt hat.

3. Verfahren zur Herstellung ines Hybridoms, welches befähigt ist, einen wie im Anspruch 1 definierten, monoklonalen Antikörper zu produ-

ner Mäuse-Myelomzelle und einer Milzzelle aus einer Maus, die mit einem Adriamycin-resistenten K562/ADM-Stamm einer myelogenen Human-Leukämiezelle K562 immunisiert worden ist, umfaßt.

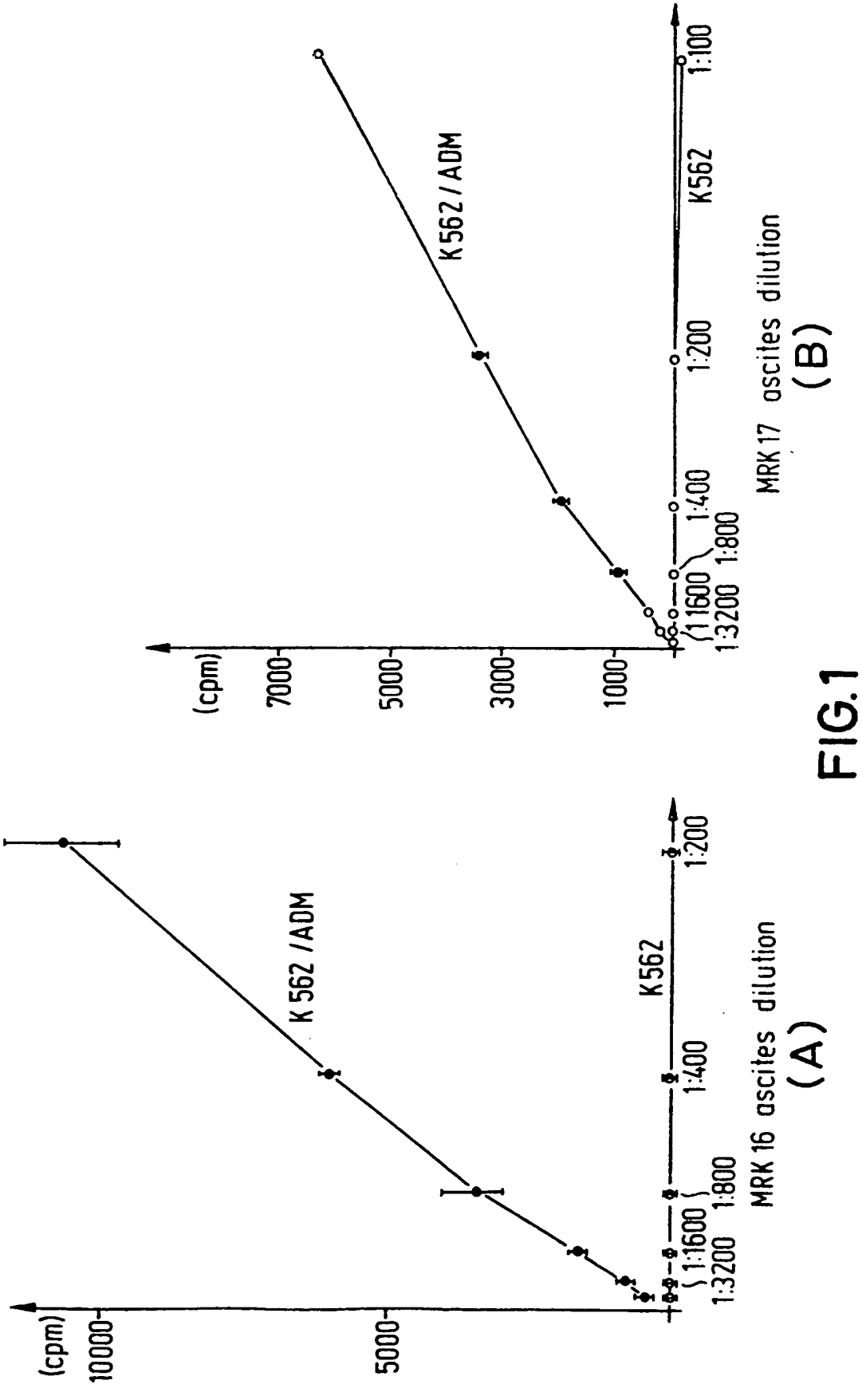

(cpm)
10000
5000
K 562 /ADM
K562
1:200
1:400
1:800
1:1600
1:3200
MRK 16 ascites dilution
(A)
(cpm)
7000
5000
3000
1000
K562 / ADM
K562
1:100
1:200
1:400
1:800
1:1600
1:3200
MRK 17 ascites dilution
(B)

FIG.1

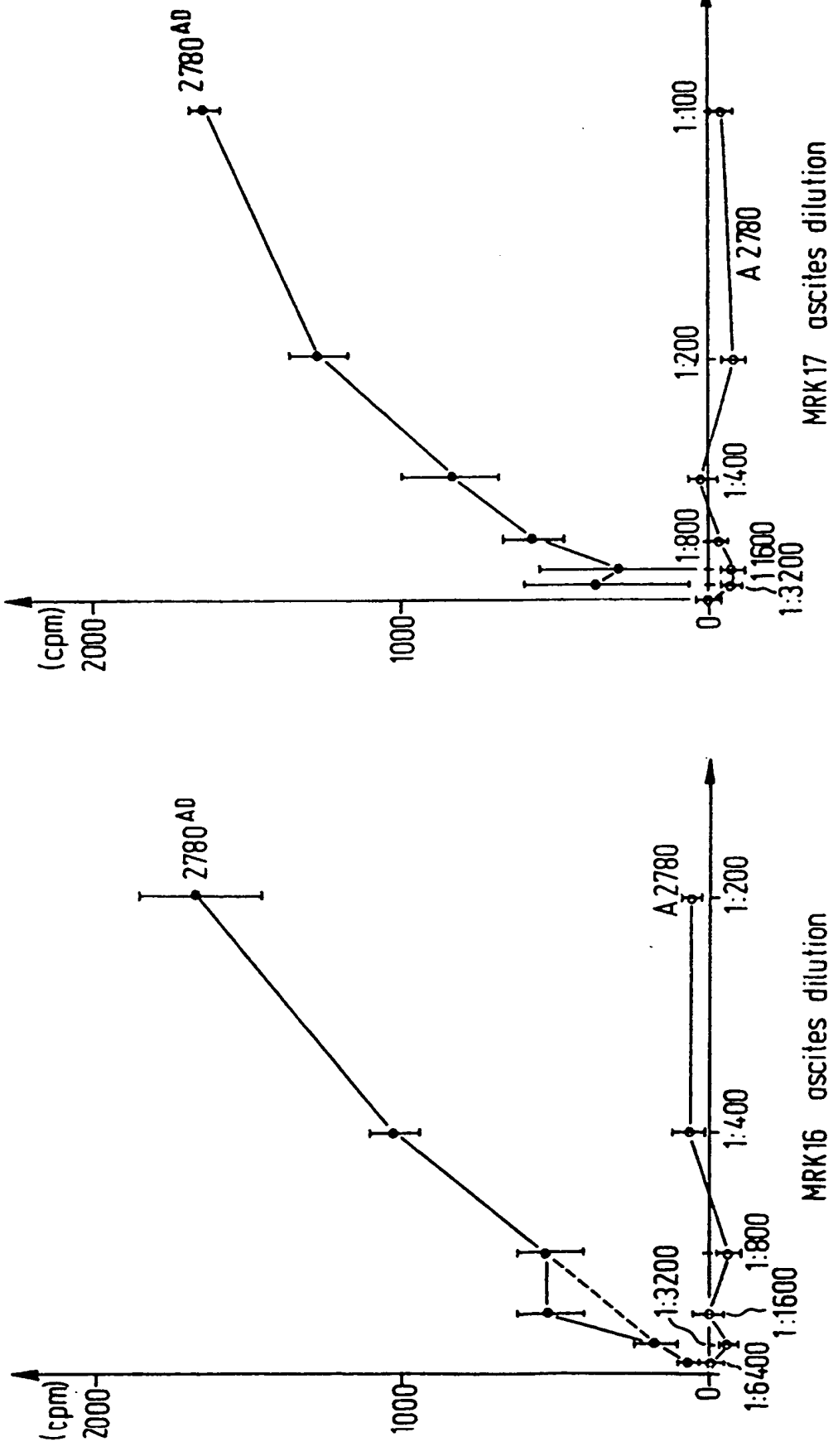

(cpm)
2000
1000
0
2780 AD
A 2780
1:100
1:200
1:400
1:800
1:1600
1:3200
1:6400
MRK16 ascites dilution
(A)
MRK17 ascites dilution
(B)

FIG.2

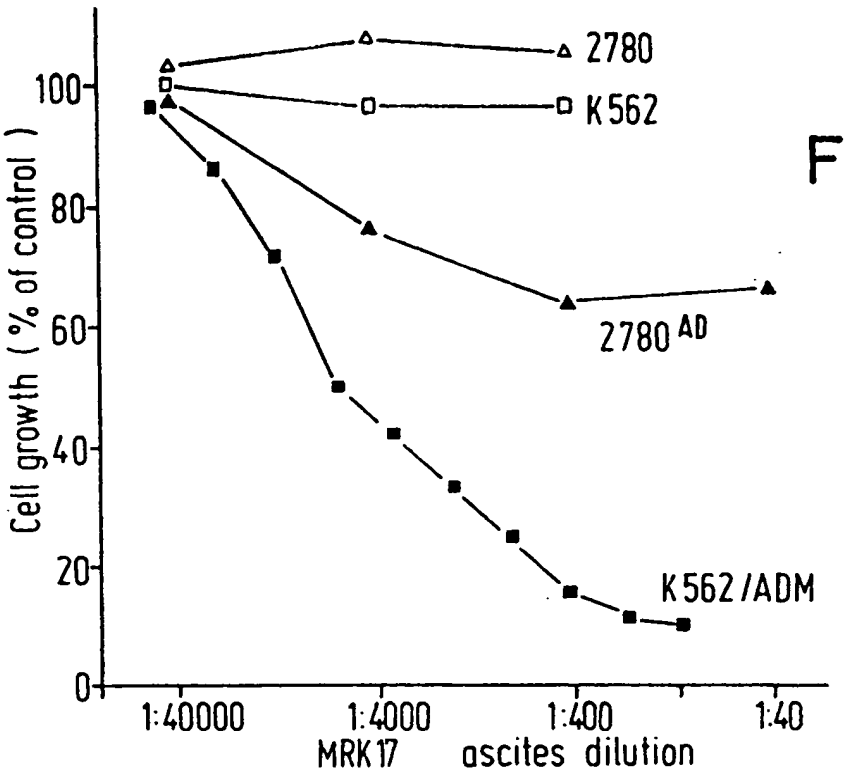

2780
K562
2780 AD
K562/ADM
Cell growth (% of control)
100
80
60
40
20
0
1:40000
1:4000
1:400
1:40
MRK17 ascites dilution

FIG.3

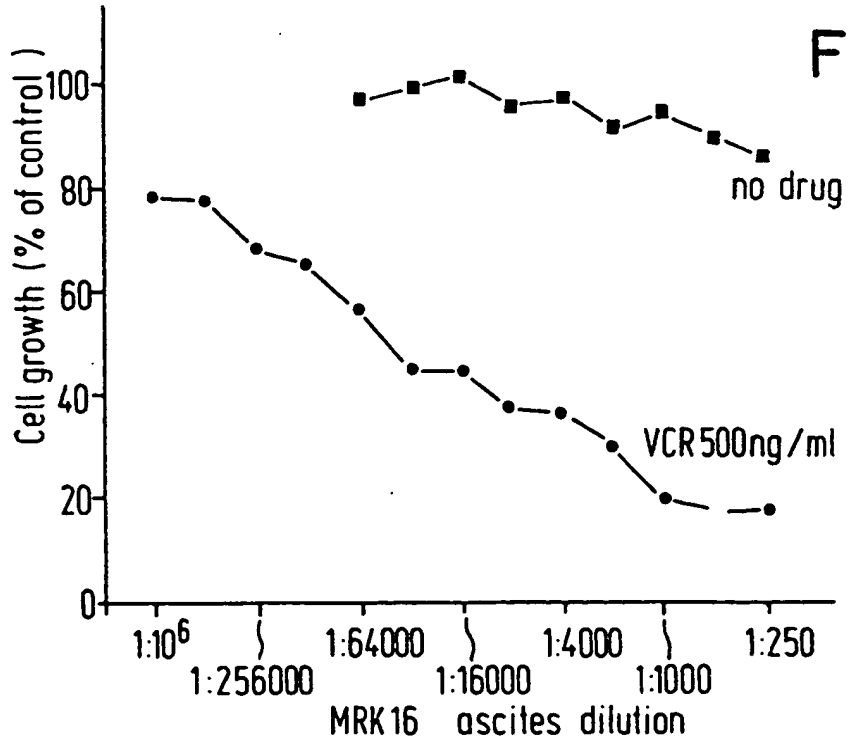

no drug
VCR500ng/ml
Cell growth (% of control)
100
80
60
40
20
0
1:10^6
1:256000
1:64000
1:16000
1:4000
1:1000
1:250
MRK16 ascites dilution

FIG.4

FIG.5

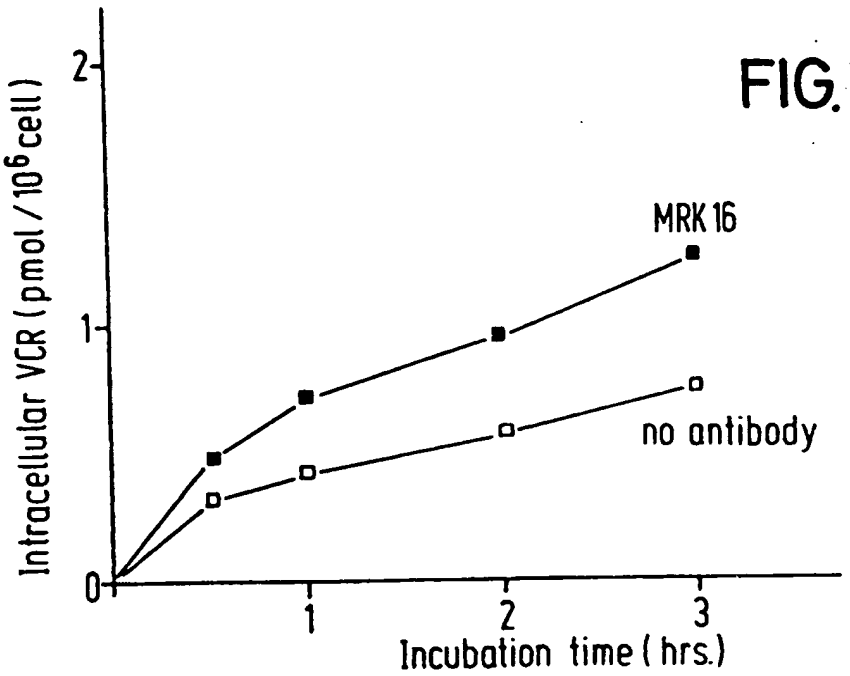
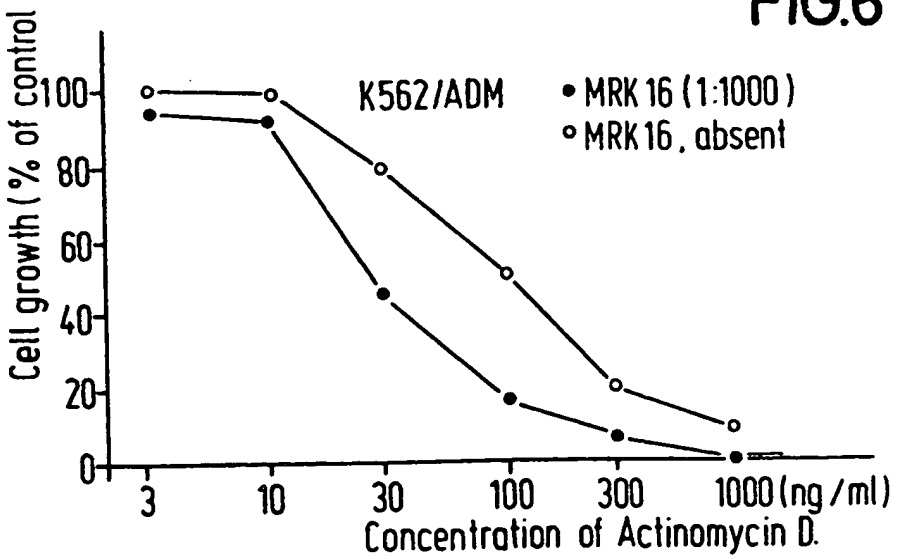

Intracellular VCR (pmol / 10^6 cell)
2
1
0
MRK 16
no antibody
1
2
3
Incubation time (hrs.)

FIG.6


Cell growth (% of control
100
80
60
40
20
0
K562/ADM
• MRK 16 (1:1000)
○ MRK 16, absent
3
10
30
100
300
1000 (ng/ml)
Concentration of Actinomycin D.